



Variations in maternal behavior in rats selected for infant ultrasonic vocalization in isolation



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ABSTRACT

Individual differences in maternal behavior in rodents are associated with altered physiology and behavior in offspring across their lifespan and across generations. Offspring of rat dams that engage in high frequencies of high-arched-back nursing and pup-licking (High-LG) show attenuated stress responses compared to those engaging in lower frequencies (Low-LG). Selective breeding also produces widespread alterations in physiology and behavior that are stable over generations. To examine processes underlying generational and developmental influences on anxiety in an animal model, we developed two lines of rats that emit either extremely high (High-USV) or low (Low-USV) rates of 45 kHz ultrasonic vocalizations in isolation at postnatal day 10. Compared to the Low-USV line, High-USV rats display increased indices of anxiety- and depression-like behavior in adulthood. The current study assessed maternal behaviors as well as oxytocin and vasopressin receptor density in High-USV and Low-USV dams to determine if selective breeding had produced differences that paralleled those found in Low- and High-LG dams. We found that Low-USV dams engage in more high-arched nursing and pup-licking than High-USV dams. Differences in oxytocin and vasopressin receptor levels were not widespread throughout the brain, with line differences in the piriform cortex and nucleus accumbens. This research illustrates the potential interplay between genetically determined (USV line) and environmental (postnatal mother–infant interactions) factors in accounting for the phenotypes associated with maternal separation induced postnatal vocalizations.

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Introduction

In the first research program to selectively breed for a mammalian infantile trait, we developed two lines of N:NIH rats that, as postnatal day (PND) 10 infants in isolation, emit either extremely high (High-USV line) or very low (Low-USV line) rates of 45 kHz ultrasonic vocalizations (Brunelli et al., 1997). The breeding design, created to model aspects of human separation anxiety, also produced alterations in the lines' physiology and behavior across development from infancy through adulthood. Compared with randomly-bred N:NIH rats, both High- and Low-USV juveniles show reduced frequency and quality of play, as well as reduced 50 kHz USV during play (Brunelli et al., 2006). High-USV adults characteristically show more anxiety-like (open field) and depressive-like (Porsolt swim) behaviors than Low-USV adults (Brunelli and Hofer, 2007). At PND18, High-USV juveniles and adults exhibit sympathetic over-activity regulating heart rate, whereas

Low-USV juveniles and adults show parasympathetic under-activation of heart rate (Brunelli and Hofer, 2007; Brunelli et al., 2002). In adulthood, Low-USV males are also more aggressive in male–male social interactions (Brunelli and Hofer, 2007). Components of female sexual behavior are reduced in the Low versus the High-USV lines, as are levels of midbrain and plasma 3α , 5α -THP (allopregnanolone), which are known to regulate sexual behavior (Frye et al., 2006; Zimmerberg et al., 2005). These phenotypic changes, based on selection for an early infant isolation-induced behavior are stable across generations (Brunelli and Hofer, 2007).

Naturally occurring individual differences in the maternal behavior of rats are also associated with lifelong variation in behavioral and neuroendocrine responses to stress and novelty in their offspring. Many, but not all, of these differences parallel those observed between the High- and Low-USV lines. Adult offspring of rat dams exhibiting high rates of maternal licking (anogenital and non-anogenital) and high-arched nursing (High-LG dams) show attenuated corticosterone responses to stress and reduced anxiety-like behavior compared to adult offspring of dams whose rates of high-arched nursing and licking are significantly lower (Low-LG dams) (Caldji et al., 1998; Cameron et al.,

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2005; Champagne et al., 2003; Francis et al., 1999). Similar to the Low-USV line, female offspring of High-LG dams show reduced sexual receptivity (Cameron et al., 2008). Play behavior is reduced in both male and female juvenile High-LG compared to Low-LG offspring, suggesting some distinction from the USV lines, where play is reduced in both Low- and High-USV individuals (Brunelli et al., 2006; Parent and Meaney, 2008). Differences in maternal behavior between High- and Low-LG dams are stable and the impact on offspring persists across generations (Francis et al., 1999).

Thus, variation in sexual behavior, anxiety, stress responses, behavioral or physiologic traits, and other phenotypes can be linked to either selective breeding for infant USV rates or to variation in the quality of early mother–infant experiences. In light of parallel effects observed in the phenotypes of High- and Low-USV lines and High- and Low-LG offspring, here we address the question of whether there are also parallel differences in the early maternal environment of the USV lines. Early in the selection process, we found that cross-fostering of pups at PND1 between High- and Low-USV line dams did not produce significantly different USV rates at PND10 compared to in-fostered pups of the same line, indicating that there was no postnatal maternal contribution to the selected behavior, USV rate (Brunelli et al., 2001). Nonetheless, the question arises as to whether maternal behaviors in the first week of lactation exist that might account for other differences between lines that emerge later in life. We hypothesized that Low-USV dams would engage in elevated levels of arched-nursing and pup-licking and thus would be comparable to High-LG dams. Investigation of the neurobiological basis of the Low- versus High-LG maternal phenotype has implicated region-specific changes in the density of oxytocin receptors in hypothalamic and limbic structures. Though the related vasopressin system has not been explored in Low- versus High-LG dams, recent evidence has demonstrated that vasopressin does promote specific forms of maternal care in rats (Bosch and Neumann, 2012). Accordingly, here we hypothesized that the presence of group differences in maternal behavior in High- and Low-USV lines would be complemented by variation in oxytocin and vasopressin receptor density in the maternal brain.

Materials and methods

Subjects and housing

All procedures used in this study were reviewed and approved by the Columbia University and New York State Psychiatric Institute's Institutional Animal Care and Use Committee. Originally, 25 breeding pairs of N:NIH rats, specifically developed for selective breeding, were provided by the NIH (Hansen and Spuhler, 1984). The phenotype selected is based on the number of USVs emitted by postnatal 10-day-old (± 1) pups in 2 min of isolation. Over many generations, all pups from all litters born were screened for isolation-induced USV rates at PND10 (± 1). In the High- and Low-USV lines respectively, individual pups vocalizing for the highest and lowest rates of vocalization within litters were selected as breeders for the next generation. The lines were maintained separately as closed breeding systems, and mating occurred only within lines. Breeders were mated with partners from litters outside the natal litter, and mating pairs did not share parents or grandparents in common (Brunelli et al., 2001). Subjects in the current study were PND90–120 females from High- and Low-USV line litters representing the 30th to 35th selected generations. Vocalization rates in these generations for the High-USV line averaged 162 calls/min ($N = 250$, range: 110–200), while Low-USV line call rates averaged 18 calls/min ($N = 348$, range: 10–23). The USV rates of adult subjects in this study were not assessed during infancy, nor were their litters. Subjects' litters were sacrificed on PND6; this obviated assessment of pup rates at the standard postnatal age of 10 days. However, litters of 9 High-USV and 8 Low-USV dams' half-siblings were tested, and were comparable to the dams themselves. A two-sample *T*-test indicated

significant differences between means of two separate distributions [$t(128) = 25.12$, $p < 0.001$]. A Kolmogorov–Smirnov Two-Sample Test ($p < 0.001$), confirmed that these two samples did not come from the same distribution. 12 High-USV and 13 Low-USV line females were bred with their respective line males between 90 and 120 days of age. At approximately gestational day 18, pregnant females were singly-housed in clear, polypropylene cages with pine bedding. Room temperature and humidity were regulated, a reversed light cycle was implemented (lights off: 0800; lights on: 1800), and food and water were available ad libitum. The day of birth was designated PND0 and litters were culled to 8–10 pups per litter, balanced by sex when possible.

Maternal behavior

Observations of maternal behavior were based on a procedure developed by Myers et al. (Myers et al., 1989). The occurrence of the following behaviors was assessed: *high-arched nursing* – dam is in a high-arched posture over pups with legs splayed; *low-arched nursing* – dam is over the litter, but is not arched and with no extension of her legs; *passive nursing* – dam is lying on her side with one or more pups attached; *licking or grooming* – dam licking the pup (either anogenital or body); *nest-building* – dam moving or carrying pine shaving bedding. A total of 12 High- and 12 Low-USV dams were observed for maternal behavior, each for five 75-minute observation periods daily for the first 6 days postpartum. Multiple observers were trained to a high level of inter-rater reliability (>0.90). Within each 75-minute observation period, the behavior of each mother was scored every 3 min (25 observations/period \times 5 periods per day = 125 observations/mother per day). Frequency of a behavior was calculated as the number of times the behavior was observed divided by the total number of observations. Total contact time with pups and a composite measure of high-arched nursing/licking and grooming were also calculated based on observed behavioral frequencies.

Oxytocin and vasopressin receptor autoradiography

Immediately following the last observation on day PND6, dams were sacrificed through rapid decapitation and brains were removed and placed briefly in liquid nitrogen, then stored at -80 °C. Brains were sectioned in the coronal plane at 20 μ m, and sections thaw-mounted onto poly-L-lysine coated slides. Slide-mounted coronal brain sections were processed for autoradiography using 125 I d(CH₂)₅[Tyr-Me]₂Tyr-NH₂] oxytocin receptor (New England Nuclear, Boston, MA) and vasopressin V1a receptor autoradiography using 125 I-lin-vasopressin [125Iphenylacetyl-D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH₂] (New England Nuclear, Boston, MA) as previously described (Champagne et al., 2001; Curley et al., 2012). All autoradiograms were analyzed using an image analysis system (MC1D-4, Interfocus Imaging, Cambridge, UK). Between three and six sections were analyzed bilaterally for each brain region. Oxytocin receptor binding was analyzed in the following regions (with respect to Bregma): (1) Dorsal lateral septum, ventral lateral septum, nucleus accumbens core, and nucleus accumbens shell (+2.52 mm to -1.92 mm), (2) Bed nucleus of the stria terminalis and posterior ventral lateral septum (+0.60 mm to -0.48 mm), (3) Medial preoptic area and ventromedial preoptic nucleus (+0.24 mm to -0.48 mm) and (4) Arcuate nucleus, central amygdala, cortical amygdala, posterior dorsal medial amygdala, intermediodorsal thalamic nucleus, posterior ventral medial amygdala, and ventromedial hypothalamus (-2.04 mm to -3.24 mm) (Paxinos and Watson, 2005). Vasopressin V1a receptor binding was analyzed in the following regions (with respect to Bregma): (1) Dorsal lateral septum, nucleus accumbens core, nucleus accumbens shell, and ventral lateral septum (+2.28 mm to +0.84 mm), (2) Interstitial nucleus of the posterior limb of the anterior commissure, lateral preoptic area, medial preoptic area, piriform cortex, septohippocampal nucleus, and septohypothalamic nucleus (+0.48 mm to -0.48 mm) and (3) Central amygdala, hippocampus,

ventrolateral thalamus, and ventromedial hypothalamus (–1.40 mm to –2.40 mm) (Paxinos and Watson, 2005). For each animal, total and non-specific binding was measured for each region and the difference taken to yield specific binding which was converted to fmol/mg using radio-labeled micro-scales (GE Healthcare). All 12 females/group were included in the analyses, with sample sizes for each brain region reduced to 9–11/group due to lack of the presence of the anatomical location on slides.

Statistics

Maternal behavioral data were collapsed across observation periods, and were analyzed as daily means using repeated-measures ANOVA with USV line as the grouping variable, and observations over the 6 postpartum days as repeated measures. Tukey's HSD post-hoc test was used in cases where a group by day interaction was determined. Receptor density data were analyzed using t-tests, with USV line as the grouping variable. Data were averaged across slide sections for each dam for each brain area examined. A false discovery rate (FDR) criterion of 5% was used to protect for the effects of multiple testing (Benjamini and Hochberg, 1995). The FDR procedure identifies the subset of p-values within which 5% should be considered as "false discoveries".

Results

Behavioral observations

Across the first 6 days of lactation, Low-USV dams spent more time in the *high-arched nursing* position than High-USV dams [Group $F(1,22) = 4.43$, $p = 0.05$; standardized mean-difference effect size = 0.4]. A strong effect of Day [$F(5,110) = 10.28$, $p < 0.001$] indicated that both lines reduced high-arched nursing over time. A significant Group \times Day interaction [$F(5,110) = 2.30$, $p = 0.05$] reflected the sharp increase in Low-USV high-arched nursing on PND3 and subsequent steeper drop over days, with group differences between lines significant at PND3 ($p < .01$) and marginally significant at PND4 ($p < .10$)

(Fig. 1A). Overall, High-USV dams spent more time in the *low-arched nursing* position [$F(1,22) = 3.60$, $p = 0.07$; standardized mean-difference effect size = 0.2], accounted for primarily by a sharp increase at Day 3, then a gradual decrease in this behavior across subsequent days [Group \times Day interaction $F(5, 110) = 2.27$, $p = 0.05$]. Group differences between lines were significant at PND3 ($p < .01$) and PND4 ($p < .05$), reflecting a reciprocal relationship to frequency of high-arched nursing. Both lines showed significant increases in low-arched nursing over Days [$F(5,110) = 4.18$, $p < 0.001$; Fig. 1B]. Frequency of *passive nursing* (Table 1) was not different between the two USV lines [$F(1,22) = 0.47$, $p = 0.50$]. Passive nursing increased in both USV lines over the six postpartum days [Day, $F(5,110) = 2.54$, $p = 0.03$] with no significant interactions [Group \times Day: $F(5,110) = 0.64$, $p = 0.67$]. Low-USV dams spent more time *licking/grooming* their pups than High-USV dams [$F(1,22) = 4.60$, $p = 0.04$; standardized mean-difference effect size = 0.5]. Both lines decreased licking over days [Day $F(5,110) = 5.13$, $p < 0.001$], with no significant Group \times Day interaction [$F(5,110) = 0.13$, $p = .89$] (Fig. 1C). Frequency of *nest-building* was not different between Low- and High-USV dams (all p 's > 0.10). Overall contact with pups did not differ by Group ($p = 0.99$); by Day ($p = 0.64$); or Group \times Day ($p = 0.67$), whereas frequency of arched-back pup licking behavior was more frequently observed amongst Low-USV dams across days [$F(1,22) = 6.0$, $p = 0.02$; standardized mean-difference effect size = 0.6; Fig. 1D]. Group means collapsed across postnatal days are shown in Table 1.

Oxytocin and vasopressin receptor density

After applying a FDR criterion of 5%, few differences were apparent in oxytocin or vasopressin V1a receptor density for dams of the two USV lines. Low-USV dams showed a trend toward greater oxytocin receptor binding than High-USV dams in the nucleus accumbens shell [$t(17) = 2.18$, $p = .05$; standardized mean-difference effect size = 0.1; Table 2]. Low-USV dams had elevated vasopressin V1a receptor binding compared to High-USV dams in the nucleus accumbens core [$t(19) = 2.93$, $p < .01$; standardized mean-difference effect size = 0.3,

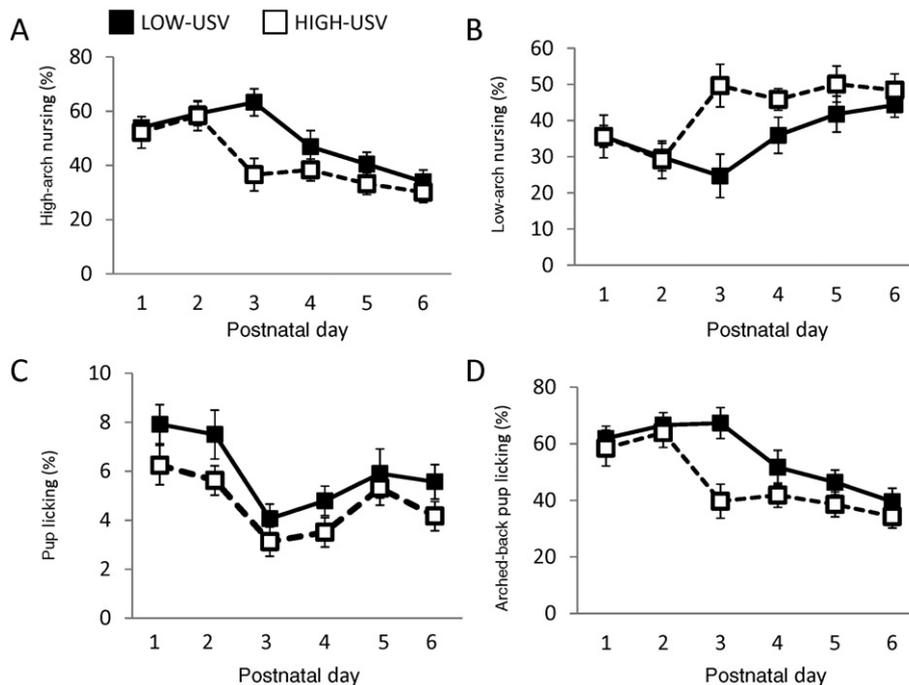


Fig. 1. Frequency of maternal behaviors over postnatal days 1–6 in High- compared with Low-USV dams. (A) A significant Group \times Day interaction reflected the sharp increase in Low-USV high-arched nursing on PND3, and subsequent steeper drop over days. (B) Overall, High-USV dams spent more time in the low-arched nursing position, accounted for primarily by a sharp increase at Day 3, then a gradual decrease in this behavior across subsequent days. (C) Low-USV dams spent more time licking/grooming their pups than High-USV dams, with no significant Group \times Day interaction. (D) Frequency of arched-back pup licking was significantly elevated in Low- compared to High-USV dams.

Table 1
Frequency of postpartum maternal behavior (%) of Low- and High-USV dams averaged across days.

	High-arched nursing*	Low-arched nursing [†]	Passive nursing	Pup-licking*	Nest-building	Contact	Arched-back pup-licking*
<i>Low-USV</i>							
Mean	49.75	35.92	3.49	6.04	1.15	90.30	44.04
SEM	2.74	2.31	0.92	0.51	0.15	2.11	2.15
N	12	12	12	12	12	12	12
<i>High-USV</i>							
Mean	41.46	43.15	5.56	4.66	1.01	92.68	36.64
SEM	2.83	3.03	2.90	0.38	0.23	1.53	2.37
N	12	12	12	12	12	12	12

[†]p < 0.10; *p < 0.05.

Table 3]. High-USV dams showed greater vasopressin V1a binding in the piriform cortex [$t(21) = 3.49$, $p < .01$; standardized mean-difference effect size = 0.4, Table 3].

Discussion

Consistent with our predictions, Low-USV line dams were observed to engage in patterns of maternal behavior similar to those reported for High-LG dams: they exhibited higher frequencies of high-arched nursing and pup-licking compared to High-USV dams. In contrast, and similar to Low-LG dams, High-USV dams were more frequently observed in the low-arched nursing position. Also consistent with studies of Low- versus High-LG dams, frequency of nest-building behavior and overall contact with pups did not differ in the two types of USV dams. Thus, selective breeding for infantile traits influenced the development of adult maternal behaviors. Though cross-fostering studies conducted between Low- and High-LG dams indicate that pup phenotype does not alter patterns of maternal LG (Champagne et al., 2003), it is unclear whether the USV phenotype of the pups or of the dam is the critical predictor of the observed differences in maternal behavior. Consistent with theories of mother-offspring coadaptation (Curley et al., 2004; Galler and Propert, 1981), it is probable that both mother and offspring contribute to this phenomenon. Cross-fostering studies using Low- and High-USV pups reared by either Low- or High-LG dams may be a strategy for exploring the interplay between pup traits and maternal behavior on subsequent neurobehavioral outcomes.

Variation in maternal behavior amongst Low- and High-USV lines could have implications for the development of phenotypes that have been documented in comparison of these lines. Though our early cross-fostering study suggested that the USV phenotype assessed at PND10 is not significantly shifted in response to changes in maternal environment, other line differences in neurobehavioral outcomes that have emerged over generations of selection in juvenile development and in adulthood may be mediated or modulated by these line differences in maternal care. Moreover, after 43 + generations of selective

breeding (Zimmerberg and Germeyan, 2015), differences in PND10 USV rates, duration and bandwidth found recently, suggest that a new cross-fostering study would be useful in parsing out selected and maternal effects. A critical consideration within these studies will be the origins of both line differences in phenotype and within-litter variation in phenotype. Studies of Low- and High-LG litters indicate significant within-litter variation in LG toward individual pups as well as within-litter variation in phenotype (Cavigelli et al., 2010; Champagne et al., 2003). It is unknown whether within-line individual differences in USV rates are related to physiological and behavioral outcomes or whether within-litter individual differences in the experience of LG is predictive of USV rates.

Analyses of region-specific differences in oxytocin receptor density in the brain reveal that unlike High- and Low-LG dams, where widespread differences in oxytocin receptor levels have been found in systems mediating maternal behavior, dams in the selected USV lines exhibited few differences in oxytocin receptor levels. We did find increased vasopressin V1a receptor levels in nucleus accumbens in Low-USV compared to High-USV dams, though this effect was localized to the core region rather than the shell. Vasopressin is expressed in the nucleus accumbens in rats (Rodríguez-Borrero et al., 2010), and increased density of vasopressin V1a receptors in the nucleus accumbens in prairie voles is associated with pair-bond formation (Wang et al., 2013). The accumbens core is rich in dopamine receptors (Zahm, 1999), suggesting a role in enhanced reward value in Low-USV dams' responses. The nucleus accumbens core is also an efferent motor component of the reward circuit (Kalivas et al., 1999; Parkinson et al., 1999), and may facilitate the motor components of maternal behavior responses to pup stimuli (Afonso et al., 2009; Barrett and Fleming, 2011). Based on the findings of the current study, we hypothesize that during the first week of lactation, the greater number of vasopressin V1a receptors in the nucleus accumbens core of Low-USV dams may engender greater activation of the nucleus accumbens core, resulting in increased processing of efferent output in associated motor pathways. The consequence of this activation is to stimulate licking/grooming behavior in Low-USV dams.

Table 2
Differences in oxytocin receptor levels in Low-USV compared with High-USV dams in various brain regions.

Brain region	Low-USV (mean ± SEM)	High-USV (mean ± SEM)	p-Value	Difference
Nucleus accumbens shell	22.4 ± 1.4	16.8 ± 1.8	0.05	Low > High
Cortical amygdala	9.8 ± 1.0	12.6 ± 1.8	0.26	
Nucleus accumbens core	8.4 ± 1.4	14.0 ± 4.2	0.26	
Posterior dorsal medial amygdala	9.8 ± 1.0	12.6 ± 2.2	0.28	
Posterior ventral medial amygdala	9.8 ± 0.4	12.6 ± 1.8	0.31	
Ventral lateral septum	16.8 ± 0.4	15.4 ± 1.4	0.45	
Ventromedial hypothalamus	12.6 ± 0.4	15.4 ± 4.2	0.48	
Medial preoptic area	12.6 ± 1.0	11.2 ± 1.4	0.60	
Dorsal lateral septum	25.2 ± 1.8	26.6 ± 0.3	0.71	
Ventromedial preoptic nucleus	12.6 ± 1.4	11.2 ± 1.0	0.73	
Bed nucleus of the stria terminalis	26.6 ± 0.3	28.0 ± 1.8	0.74	
Central amygdala	30.8 ± 3.6	32.2 ± 3.6	0.82	
Posterior ventral lateral septum	18.2 ± 1.8	18.2 ± 0.3	0.88	
Intermediodorsal thalamic nucleus	16.8 ± 1.4	15.4 ± 1.8	0.92	
Arcuate nucleus	22.4 ± 0.3	22.4 ± 0.3	0.97	

Table 3
Differences in vasopressin V1a receptor levels in Low-USV compared with High-USV dams in various brain regions.

Brain region	Low-USV (mean ± SEM)	High-USV (mean ± SEM)	p-Value	Difference
Piriform cortex	23.8 ± 0.8	28.0 ± 1.3	0.00*	High > Low
Nucleus accumbens core	19.6 ± 0.8	16.8 ± 0.8	0.01*	Low > High
Lateral preoptic area	21.0 ± 0.8	23.8 ± 1.3	0.04	
Medial preoptic area	11.2 ± 0.8	14.0 ± 1.3	0.07	
Hippocampus	29.5 ± 2.1	33.6 ± 2.1	0.10	
Nucleus accumbens shell	18.2 ± 0.8	15.4 ± 1.3	0.17	
Ventromedial hypothalamus	9.8 ± 0.8	11.2 ± 2.1	0.31	
Central amygdala	37.8 ± 3.4	33.6 ± 3.4	0.36	
Septohypothalamic nucleus	18.2 ± 0.8	19.6 ± 1.7	0.36	
Ventral lateral septum	29.4 ± 2.5	26.6 ± 1.7	0.49	
Ventrolateral thalamus	30.8 ± 2.1	29.4 ± 2.9	0.56	
Interstitial nucleus of the posterior limb of the anterior commissure	30.8 ± 1.7	32.2 ± 0.8	0.69	
Septohippocampal nucleus	22.4 ± 2.1	23.8 ± 3.8	0.85	
Dorsal lateral septum	33.6 ± 2.5	33.6 ± 3.8	0.96	

* p-Values pass the 5% FDR threshold.

Interestingly, we identified a greater number of vasopressin V1a receptors in piriform cortex in the High-USV dams compared to Low-USV dams. Although the piriform cortex is generally thought to function as a “primary” cortex for olfaction, it functions as an association cortex as well, having reciprocal connections with other primary sensory areas and with a number of cortical areas and the amygdala, suggesting that it is processing multisensory information before sending it to other structures (Johnson et al., 2000). In maternal mice, Koch and Ehret (Koch and Ehret, 1991) showed that lesions to the piriform and entorhinal cortex reduced appetitive responses (searching) to pup auditory cues, but not olfactory cues that directed consummatory behavior (retrieving). Geissler et al. (Geissler et al., 2013) recently used c-fos labeling to show that the piriform cortex was differentially active in maternal mice as they heard familiar and unfamiliar mouse pup ultrasounds. In the current context, greater vasopressin V1a receptor activity in High-USV line dam piriform cortex may reflect greater maternal responses to High line pup USV, or some unknown sensory stimuli in High line pups.

Based on the current study, it is apparent that selective breeding for high and low rates of infant vocalization in response to maternal separation and isolation in a novel environment has produced two distinct patterns of maternal behavior in adult females of the two lines. In this respect, the High- and Low-USV lines share maternal traits with other selectively bred animal models that were selected as adults for ‘high’ or ‘low’ behavioral or physiological indices of anxiety/depression or of aggression/impulsivity (e.g. (Bosch and Neumann, 2012; Myers, 1992; Neumann et al., 2010)). In Spontaneously Hypertensive rats (SHR) originally bred for essential hypertension (Wultz et al., 1990), the dams, like High-LG and Low-USV dams, showed significantly higher levels of high-arched nursing and pup-licking than normotensive Wistar-Kyoto (WKY) dams (Myers et al., 1989). It should be noted that the group differences in maternal behavior of Low- versus High-USV dams are relatively modest compared to those in the LG model and this may account for the limited number of neurobiological differences between lines in oxytocin and vasopressin V1a receptor density. However, not all variations in maternal behavior are linked with adult phenotypes in their offspring, nor are central mechanisms the same in different lines, strains or species (Champagne et al., 2007). For example, in rat lines selected for high levels of anxiety as adults (High Anxiety Behavior, HAB rats), dams exhibit higher levels of high-arched nursing and licking/grooming than dams of rat lines selected for low levels of anxiety (Low Anxiety

Behavior, LAB); thus, their maternal behavior is not correlated with their adult phenotype (Bosch and Neumann, 2008). It is clear that the relationship between anxiety-like/depressive-like behaviors and maternal behavior is complex, requiring further exploration of the neuroendocrine basis of these complex behavioral phenotypes (Curley et al., 2012).

Conclusions

Taken together, our findings suggest that differences shown in maternal behavior between High- and Low-USV dams are supported by relatively few differences in expression of oxytocin and vasopressin receptors as targets of selection in the USV lines. No differences were found in core structures mediating maternal care in the rat. Differences were found in the piriform cortex mediating sensory information processing in High-USV dams, and in the nucleus accumbens shell and nucleus accumbens core in Low-USV dams, mediating memory and motivational systems, and motor systems that may be consistent with differences in pup licking (Numan and Stolzenberg, 2009). Thus, modulation by sensory and emotional information processed by these and associated structures may have altered both input and output pathways in the expression of maternal behavior. The resulting flexibility may enable dams to respond to, as well as influence changes in offspring behavior and physiology.

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